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Some Characteristics of Ventilation and Coughing in the Bluegill 'Lepomis macrochris' Rafinesque

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SOME CHARACTERISTICS OF VENTILATION AND COUGHING IN THE BLUEGILL *LEPOMIS MACROCHIRUS* RAFINESQUE

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ABSTRACT

Simultaneous measurement of buccal water-pressure changes verified the presence of coughing manoeuvres on strip-chart recordings of bioelectric potentials associated with respiratory movements and sensed with non-contact electrodes from cannulated bluegills. Additional recordings, here called 'electrobranchiograms' (EBGs), showed that non-cannulated and well-acclimated bluegills normally display an arrhythmic respiratory pattern similar to Biot's breathing, in which pauses in breathing (apnea) characteristically alternate with groups of ventilating cycles. Three types of bluegill coughing manoeuvres were identified: type I, the most common form, were large amplitude coughs due to an interruption and momentary reversal of developing bioelectric potential of a ventilating cycle followed by a substantial burst in potential; type II, a quick spike-like cough, occurred as a momentary pause in potential followed by a burst and type III coughs were an interruption and reversal, usually near the peak of a ventilating cycle and without the accompanying burst in potential. The cough occupied from about one-seventh to one-fifth of a normal ventilating cycle as seen on an EBG and occurred 1·2 ± 0·6 times a minute in well-rested fish. Enhanced amplitude relative to adjacent ventilatory cycles (= 1·5 times) was the most important factor in determining when acclimated bluegills coughed, but coughing amplitude often diminished during exposure to zinc sulphate even though the depth of ventilation appeared to increase substantially.

INTRODUCTION

Coughing in fish has a weil-documented physiological basis. Kuiper (1907) and Bijtel (1949) were among the first to give detailed descriptions of coughing movements in fish but, more recently, other workers have described the muscular, skeletal, neurological and hydrokinetic events that occur within a fish during coughing and other respiratory incidents (Hughes & Shelton, 1958; Shelton, 1959,

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1970; Saunders, 1961; Pasztor & Kleerekoper, 1962; Hughes, 1964, 1975; Ballintijn, 1969; Satchell & Maddalena, 1972; Young, 1972; Hughes and Morgan, 1973; Capra, 1975; Holeton & Jones, 1975; Ballintijn & Roberts, 1976; Ballintijn & Alink, 1977; Hughes & Adeney, 1977 and Rovainen, 1977). Using cannulated fish and manometric techniques in most instances, they showed that some of the most definitive indications that a fish had coughed were associated with the water-pressure waveforms generated within the buccal and opercular cavities, and described the cough as an interruption of the normal ventilating cycle with a more rapid expansion and contraction of the buccal and opercular cavities that functioned primarily to clear the gills of accumulated debris.

Coughing in fish has also drawn considerable attention as a promising sublethal bioassay since Schaumberg *et al.* (1967) first applied these techniques to aquatic toxicological research as a method for measuring the effects of pollutants on fish respiration. Research to date, reviewed in part by Drummond & Carlson (1977), has shown that, besides occurring naturally in a variety of fish species as a gill-clearing mechanism, coughing responses (1) are elicited by chemical as well as mechanical stimuli; (2) increase quickly, usually within hours after first exposure; (3) are usually proportional to sublethal toxicant concentration and (4) are predictive of long-term adverse effects at levels near the maximum acceptable toxicant concentration (MATC) for several single toxicants so far investigated. For these reasons, and the intriguing possibility that a fish can integrate the complex interrelationships of several possible toxicants and antagonistic substances present in a waste into a single well-defined response, the potential exists for developing biomonitoring systems similar to those proposed by Cairns *et al.* (1973) and Morgan (1975, 1977) that are capable of rapid assessment of treated complex industrial wastes, but based on changes in cough frequency (Carlson & Drummond, 1978) or changes in both ventilation and coughing frequencies rather than on changes in ventilation rate only.

The implementation of any biomonitoring system, however, requires bioassay procedures and techniques that are readily amenable as a routine laboratory and field method. Although cannulated fish yield a great deal of information about respiratory events in fish, manometric techniques are too difficult to use as a routine device for the rapid monitoring of industrial effluents, especially where several fish and long monitoring periods are required. Alternatively, electrode chambers similar in design to those of Kleerekoper & Sibakin (1956), Camougis (1960, 1970), Spoor *et al.* (1971), Rommel (1973), Strange *et al.* (1975), Rosseland (1976), Marshall & Snyder (1977), Yakimovich & Bergman (1977), or Gruber *et al.* (1979) seem ideally suited to this task. These chambers incorporate unimplanted electrodes to detect bioelectric potentials associated with the respiratory movements of a confined, but otherwise unrestrained, fish. These signals, of the order of 0·01–400 μV (Roberts, 1964; Barham *et al.*, 1969), are amplified and sometimes filtered, are usually displayed on a polygraph recorder, and, except for a measurement of ventilation

amplitude (Heath, 1972), provide essentially the same information about respiratory movements as do strip-chart recordings of water-pressure waveforms.

As adaptable as the electrode chamber may be, the method is not without some inherent difficulties. Another requirement of any biomonitoring system is that the data generated be presented in a way readily assimilable by the technicians expected to run the system. Strip-chart recordings made with non-contact electrodes presumably represent all the measurable bioelectric potentials occurring within the fish, and these may therefore arise from numerous sources other than ventilating movements (e.g. fin movements or other locomotor activity.) Because these potentials may obscure the record or, in some cases, may imitate coughing movements, it is difficult at times to segregate coughs with certainty as they appear on the strip-chart recording. Although experience acquired through interpretation of numerous strip-chart records in conjunction with visual observation of the fish diminishes the difficulty, the uninitiated who desire to analyse fish-cough data may find that they can only do so on less than objective terms.

The main objective of this research was to alleviate some of this difficulty by defining the ventilatory characteristics of the bluegill *Lepomis macrochirus* Rafinesque as measured with non-contact electrodes during both normal respiration and toxicant stress. The bluegill was selected for this study because it is a ubiquitous species, the species is frequently used for effluent testing, and strip-chart recordings of this fish sometimes show an irregular ventilating pattern punctuated by coughs that are hard to discern. The research was designed in two concurrent phases. The first phase was the verification of the ability to distinguish fish coughs from the various bioelectric potentials detected by external electrodes. This was accomplished by correlating coughs recorded as biopotentials with those recorded simultaneously as another distinct physiological event, i.e. water-pressure changes within the buccal cavity of a cannulated fish. The second phase was the determination of the definitive features of coughs recorded as biopotentials from free-swimming fish. Because little information exists on the waveform generated in the bluegill during coughing manoeuvres, detailed measurements of several ventilating characteristics may provide some clues as to what constitutes coughs on a strip-chart record. Also, representative recordings of the various ventilating and coughing movements of a bluegill and the frequency of such events under controlled conditions and during sublethal and lethal toxicant stress will serve as a guide for those contemplating the use of bluegills in a biomonitoring programme based on a respiration bioassay.

METHODS AND MATERIALS

The test system included two electrode chambers similar to that developed by Spoor *et al.* (1971). Each chamber, 20 cm long × 5 cm wide × 5 cm high, was constructed of glass, silicon glue, and stainless steel wire electrodes. A glass partition narrowed one

chamber to less than 5 cm to prevent cannulated fish from turning around and twisting their cannula. One electrode was glued to the drain end, and the other was mounted on a glass baffle located 5 cm from the water inlet. The electrodes were shielded below water level with small sections of tygon tubing fitted 3 mm apart so that the fish could not touch the electrodes and interfere with the signal being picked up. Patch cords connected each electrode to a junction box, and from there a shielded two-conductor cable led to high-gain condenser-coupled preamplifiers set to a time constant of 0·3. The amplified respiratory signals were recorded on a rectilinear strip-chart recorder.

Acclimation water or toxicant-laden water was delivered to each electrode chamber by an airlift system modified after that described by Carlson & Drummond (1978). Briefly, each electrode chamber rested on a glass tray divided vertically by two baffles into three compartments. The baffles were shorter than the tray length by 3 mm on alternate ends so that water flowed around the end of each baffle into the next compartment. During flow-through toxicity tests toxicant was metered from a stock bottle via constant-flow capillary tubing into the first compartment, along with the incoming water. To promote mixing, air was fed through a glass tube into the second compartment and turned the water over. From the third compartment airlift tubes lifted the acclimation water or constant toxicant concentration into the electrode chamber at 50 ml min^{-1}. Excess water flowed out of the glass tray through a drain hole located 10 cm from the bottom, and the electrode chamber drained outside the glass tray.

The exposure apparatus so far described was placed in a 'viewing chamber'. Because the bluegill is sensitive to disturbance (Heath, 1972) one-way glass was used to prevent changes in ventilation rates or ventilating patterns by the fish due to their reaction to the presence of an observer. The viewing compartment was a 56-litre all-glass aquarium with one-way glass glued to one side; the remaining three sides were shielded with styrofoam. A wooden cover had holes drilled in it for entrance of necessary water supply, air, and toxicant lines and for exiting cannula and electrode patch cords. A fluorescent light mounted in the cover provided a high level of light within the compartment, and background illumination was reduced so that fish could not see to the outside. Diel respiratory patterns observed in bluegills (Sparks *et al.*, 1972) were almost eliminated by a 24-h photoperiod.

Lake Superior water used for holding and acclimation of the test fish had a pH of $7·5 \pm 0·2$ and a total hardness of 44–46 mg $litre^{-1}$ (as $CaCO_3$). The water was aerated in a series of reservoirs to maintain dissolved oxygen close to air saturation. Temperature was adjusted to $22° \pm 1·0$ °C and the water was delivered to the glass trays at 100 ml min^{-1}.

Juvenile bluegills were obtained from the Lake Mills National Fish Hatchery and were held in 37-litre glass aquaria. The average length was $130 \pm 8·4$ mm; average weight was $35·3 \pm 7·1$ mg. The fish were maintained at the test temperature and photoperiod for several months before they were used and were fed frozen brine shrimp and a pelletised trout food.

Test fish were cannulated according to the method of Saunders (1961). The fish were anaesthetised in 100 mg MS-222 litre $^{-1}$ and a 20-gauge hypodermic needle was used to pierce the fish's snout just behind the oral valve and midway between the anterior nares. A length of 0·86 mm inside diameter polyethylene tubing (PE90) was sharpened to a point and inserted through the puncture into the fish's mouth. The cannula was snipped, heat-flared at the tip, and pulled back tight against the roof of the mouth. The cannulated fish was placed in its electrode chamber, and the cannula was filled with distilled water by gentle depression of a 20 cc syringe mounted on a three-way stopcock. A turn of the stopcock connected the cannula to a Statham P23BB pressure transducer. A strain-gauge coupler fed the converted signal into an amplifier, and the amplified signal was recorded simultaneously with that signal originating from the external electrodes.

Once the cannulated fish was in its chamber, a free-swimming fish was placed in the adjacent electrode chamber. Both fish were allowed to acclimate for 72 h before exposure to the toxicant. Nominal toxicant concentrations were used because the objective of this research was only to define ventilating and coughing patterns, and not to quantify a response in precise terms to a particular toxicant. Zinc (as $ZnSO_4$) is well known to stimulate coughing in fish and has a 48-h LC_{50} value of 5·46 mg of Zn per litre for bluegills in soft water (Pickering & Henderson, 1966). Fish in this series were tested at 3·0 and 10.0 mg of Zn per litre as representative sublethal and lethal concentrations, respectively.

Both the cannulated and free-swimming fish were monitored for ventilating and coughing patterns at 1, 24, 48, and 72 h during acclimation and at 2, 4, 8, 24, and 48 h during exposure. Strip-chart recordings were usually made for 1 h during these times, and normally at a paper speed of 0·1 cm s $^{-1}$. Because the optimum paper speed for making measurements of the various ventilating and coughing movements was not known at the beginning of these tests, recordings were also periodically made at 0·25, 0·5, 1·0 and 2·5 cm s $^{-1}$ during the monitoring periods. Frequently, the fish were observed and coughs or other anomalous respiratory events were noted with a remote marker.

The 1-h strip-chart recording for each period was divided into twelve 5-min segments. Coughs were counted for each segment and expressed as coughs per minute, and a representative 1-min portion from each segment was used to determine the ventilation rate per minute. Several other characteristics associated with ventilation and coughing were measured and are presented, along with the basis for measuring them, in the Results section.

RESULTS

Bioelectric potential waveform

Simultaneous recordings of buccal-pressure and bioelectric potential waveforms showed a one-to-one correspondence of all ventilating movements in twenty

cannulated bluegills (Fig. 1). For the most part buccal pressure developed similar to that described by Hughes (1975) for rainbow trout, except that, relative to positive pressure cycles, the bluegills did not develop as much negative pressure during coughs. However, the development of any negative pressure after the start of a ventilating cycle was such a dramatic departure from the normal waveform that coughing movements were clearly delineated on the buccal-pressure recording. Although coughs were not so clearly defined in some instances on the bioelectric potential recording, their presence was nevertheless verified. Ventilation and coughing frequencies of cannulated and free-swimming fish are given in Table 1.

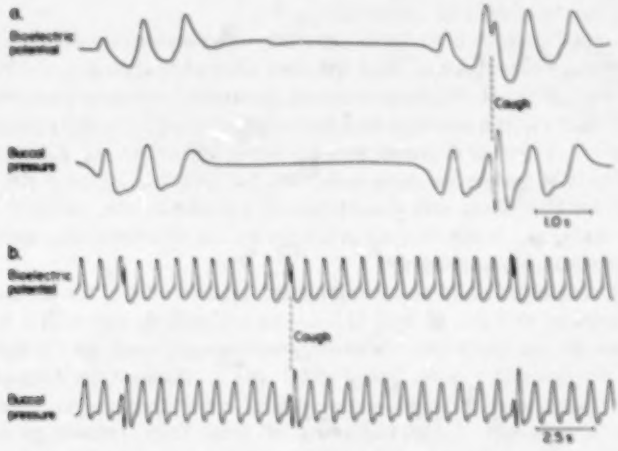

Fig. 1. Simultaneous recordings of bioelectric potentials associated with respiratory movements and buccal water pressure changes in the bluegill. (a) Acclimated fish; (b) at 1-h exposure to 10 mg $ZnSO_4$ $litre^{-1}$.

The breathing signals obtained from twenty-five non-cannulated, free-swimming bluegills showed that normally the fish did not ventilate continuously but in groups of ventilating cycles separated by periods of apnea. Duration of these pauses was measured most conveniently on the strip-chart records taken at 0·5 and 1·0 cm s^{-1}. Other measurements, including duration of the ventilating cycles and coughs and the amplitudes of both, were best made on records obtained at 2·5 cm s^{-1}.

Ventilating cycles of acclimated bluegills were typically biphasic and bicomponential, except for the first cycle after apnea (Fig. 2). This cycle often began as a slight drop in potential from baseline followed by a relatively quick, singular component signal to peak potential or, in some instances, simply occurred as a positive, singular component signal above baseline. The positive peak of this and all

succeeding cycles in a group was followed by a relaxation in potential that invariably became negative relative to the baseline. Duration of the ventilating cycle was determined as follows. The horizontal distance between the first deviation from baseline to the most negative point after peak potential constituted the duration of the first cycle. Thereafter, each cycle was timed as the distance between the most negative points on either side (Fig. 3). Because chart speed was a constant 2·5 cm s^{-1}, these distances were easily converted to time in seconds. Ventilating amplitude was obtained by measuring the vertical distance from the most negative point in each cycle to the peak of positive potential.

TABLE I

VENTILATION AND COUGHING FREQUENCY OF CANNULATED AND FREE-SWIMMING BLUEGILLS DURING THE ACCLIMATION PERIOD AND DURING EXPOSURE TO 10 mg $ZnSO_4$ litre^{-1}

Time	*Cannulated*		*Free-swimming*	
	Ventilations min^{-1}	*Coughs min^{-1}*	*Ventilations min^{-1}*	*Coughs min^{-1}*
Acclimation period (h):				
1	69 ± 16[a]	3·5 ± 2·2	50 ± 22	2·8 ± 1·2
24	34 ± 12	2·4 ± 2·5	19 ± 6	1·3 ± 0.6
48	29 ± 11	1·9 ± 1·2	18 ± 4	1·2 ± 0.6
72	28 ± 12	2·0 ± 1·6	17 ± 4	1·2 ± 0·6
Exposure period (h):				
2	50 ± 19	6·2 ± 1·3	30 ± 10	5·1 ± 1·8
4	55 ± 31	12·0 ± 7·4	39 ± 25	10·8 ± 10·0
8	80 ± 11	25·8 ± 1·5	76 ± 32	15·9 ± 5·2
24	82 ± 17	17·4 ± 4·0	76 ± 22	17·6 ± 4·1
48	—[b]	—[b]	75 ± 4	—[c]

[a] Mean ± standard deviation.
[b] No cannulated fish survived 48 h.
[c] Cough data unavailable.

Although gradations existed, bluegill coughs occurred in three primary patterns designated as types. The most common form of coughing manoeuvre, type I, was a momentary cessation of bioelectric potential during the developing ventilatory cycle and usually occurred at, or before, the peak of potential (Fig. 3a). The signal reversed itself during this period of inactivity and at times would drop below the normal baseline. The cessation was succeeded by a quick burst in potential and the overall effect was an increased amplitude of that ventilating cycle compared with neighbouring cycles. Type I cough duration was measured in one of two ways: if it occurred before or at the peak, the cough was the horizontal distance between the first point of cessation and the peak: or, if it occurred after the normal positive peak, duration was the distance brtween the lowest point of the relaxing potential, which was almost always above baseline, and the second peak of potential.

Occurring less commonly, but still frequent in some fish, were type II coughs. This

cough was a very rapid, spike-like burst in potential preceded by a momentary, sometimes almost imperceptible, pause in the developing potential (Fig. 3b). Without the accompanying reversal in signal, this pause presented itself as a slight shoulder on the ventilatory movement that was usually closer to the beginning of a cycle than the peak. Duration in this event was the horizontal distance between the leading edge of the pause and the peak.

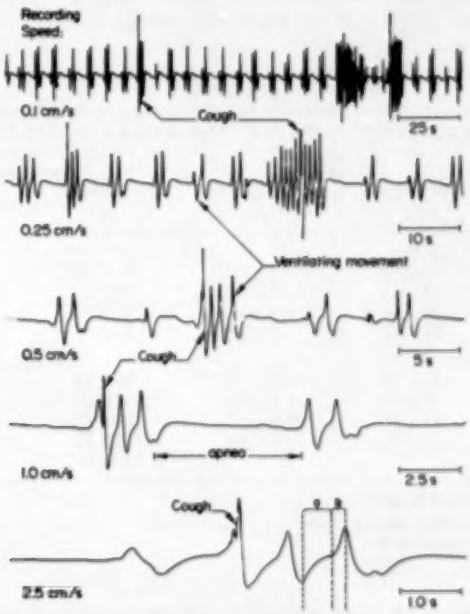

Fig. 2. Respiratory pattern of an acclimated (72-h) bluegill recorded at different strip-chart paper speeds. In the tracing made at 2·5 cm s^{-1} (a) and (b) indicate the two major components of positive potential most commonly seen in a typical ventilating cycle.

Type III coughs differed from either of the above in that they occurred almost exclusively during periods of hyperventilation in lethally exposed fish. This cough typically occurred at, or near, the peak of a ventilatory cycle, appeared mainly as a momentary cessation with little accompanying reversal in the signal, and very seldom showed a significant burst in potential after the cessation (Fig. 3c). Measurement of cough duration was similar to that for a type I cough, and in all types of coughing amplitude was the vertical distance from the bottommost point during cessation or pause to the peak in potential for that ventilating cycle.

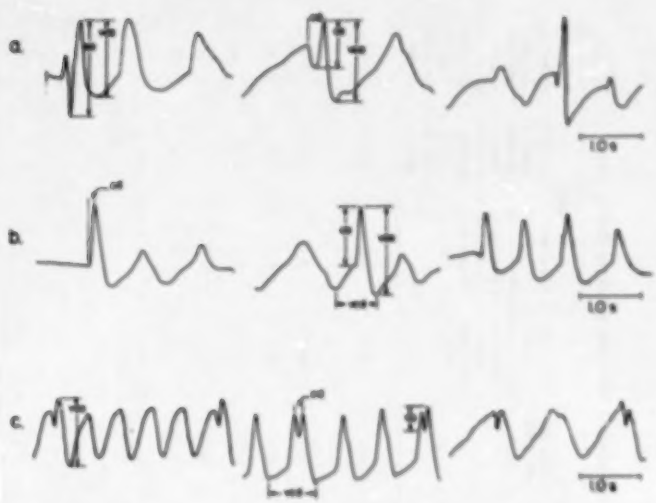

Fig. 3. Three coughing manoeuvres commonly found in the respiratory patterns of the bluegill and designated as (a) type I, (b) type II, and (c) type III coughs. Basis for amplitude and duration measurements are cough amplitude (ca); ventilating cycle amplitude (vca); cough duration (cd) and ventilating cycle duration (vcd).

Some problems were associated with measurements of amplitude. For instance, signal amplitude depended for the most part on amplifier settings, and the settings were not normalised between fish or between treatments for the same fish. Direct comparisons of ventilating or coughing amplitude could not, therefore, be made between fish or between treatments. Nevertheless, for any given fish and treatment, the relationship between cough amplitude and the amplitude of the entire ventilatory cycle in which it occurred or between the amplitude of cough-containing cycles and those adjacent to it was conceivably constant. If this were so, these constants could be examined in terms of what constitutes a normal cough-to-ventilatory-cycle ratio or cough-containing-cycle-to-adjacent-ventilatory-cycle ratio and what effect a toxicant would have on these interrelationships.

Respiratory patterns

Most of the results presented in the following sections are summarised in Table 2.

Acclimation: Free-swimming bluegills were considered fully acclimated after 72 h in their chambers; two fish held 120 h and not exposed to any toxicant showed no further decline in cough and ventilation frequencies. During acclimation, ventilation declined from 50 min^{-1} after 1 h to 17 at 72 h, and cough frequency dropped from 2·8 to 1·2 min^{-1}, respectively (Table 1). Most of the decline occurred

TABLE 2

RESPIRATORY CHARACTERISTICS OF BLUEGILLS AFTER 72-h ACCLIMATION AND DURING EXPOSURE TO 10 mg $ZnSO_4$ LITRE^{-1}

Time	Apnea		Cough	Duration (ms)		Amplitude (mm)		Ratio[a]			
	Duration (s)	Number of ventilatory cycles following apnea		Cough-containing cycle	Adjacent ventilatory cycles	Cough	Cough-containing cycle	Adjacent ventilatory cycles	Duration cough / Duration cough-containing cycle	Amplitude cough / Amplitude cough-containing cycle	Duration cough-containing cycle / Duration adjacent ventilatory cycles	Amplitude cough-containing cycle / Amplitude adjacent ventilatory cycles
Acclimation period (h)												
72	3.3 ± 2.6[b]	2.7 ± 1.9 (2)[c]	109 ± 63 (120)[d]	830 ± 385 (725)	940 ± 385 (870)	10.0	30.5	19.6	0.10	0.61	0.82	3.03
Exposure period (h)												
2	0.3 ± 2.1	2.0 ± 1.0 (2)	116 ± 30 (102)	655 ± 170 (590)	740 ± 190 (710)	6.0	20.6	13.1	0.17	0.39	0.82	1.56
4	3.9 ± 2.6	0.9 ± 1.1 (0)	99 ± 28 (86)	710 ± 170 (640)	827 ± 173 (797)	8.0	24.0	22.5	0.14	0.33	1.00	1.10
8	–[e]	—	131 ± 22 (128)	880 ± 320 (790)	930 ± 288 (820)	6.0	20.4	20.0	0.16	0.29	0.90	1.02
24	—	—	107 ± 29 (100)	767 ± 169 (725)	825 ± 122 (794)	6.0	20.4	15.0	0.14	0.29	1.22	1.29
48	—	—	89 ± 9[f] (86)	648 ± 36 (630)	851 ± 24 (851)	9.1	18.0	17.1	0.12	0.45	0.80	1.04

[a] Ratios calculated from grand geometric means.
[b] Mean ± standard deviation.
[c] Grand geometric means.
[d] Mode.
[e] Apnea no longer present.
[f] One fish only.

within the first 24 h. By 72 h acclimation locomotor activity was minimal, the fish was resting quietly in its chamber and respiratory patterns, although variable from fish to fish, were smooth and regular (Fig. 2).

Apnea was present in all acclimated fish and averaged 5·3 s. The mean number of ventilating cycles after each apneic period was 2·7 with a mode of two; the latter occurred 195 times in 430 observations. The second most prevalent number of cycles was three, followed by one and four cycles, and only rarely did the number of cycles in any one group exceed five. Coughs never appeared in a lone ventilating cycle, and the mode for those groups containing coughs was three cycles. Of all the groups of ventilating cycles examined from twenty-three fish, 7 % contained coughs.

The average ventilating cycle lasted 944 ms, and the coughs, most of which were large in acclimated fish and similar to that in Fig. 2a, averaged 169 ms. The ratio relating its duration to that of the ventilating cycle in which it occurred was 0·18, or approximately one-fifth of a normal cycle. As for amplitudes, a measure of that portion of the ventilating cycle ascribed to the cough showed that it produced, on average, 0·61 of the total change in potential. Overall, amplitudes of those cycles containing coughs averaged 1·55 times greater than the amplitudes of adjacent cough-free ventilating cycles.

Sublethal exposure to $ZnSO_4$: Three fish only were exposed to 2·7 mg $ZnSO_4$ litre^{-1}, and not enough records were obtained to determine typical bluegill respiratory characteristics when fish are subjected to that level of this toxicant. However, Fig. 4 shows the ventilatory responses from one of the fish at 72 h into the exposure, and the pattern was one typically seen in previous sublethal exposures. Ventilation and coughing rates had increased from those of an acclimated fish to 69·5 and 10·4 min^{-1}, respectively, within 2 h after the start of exposure. Ventilation rate declined, however, to approximately 40 min^{-1} by the end of 72 hours' exposure, while cough rate declined to about 5 min^{-1}.

Except for a period of continuous breathing in one fish during the first 2 h after the start of exposure, apneic periods were present throughout the sublethal tests. At 24 h exposure, 54 % of the ventilating groups contained coughs, and at 48 h exposure coughs were found in 100 % of the groups examined. By 72 h this value had declined to 24 %.

Coughing and ventilatory movements were quicker in exposed fish than in acclimated fish. For instance, at 48 h into exposure, the coughs averaged 72 ms in duration, the cycle containing a cough was timed at 513 ms, and adjacent ventilatory cycles were 634 ms long. For all exposure periods the cough-to-ventilatory-cycle ratio was 1:7 (0·14), and the coughs contributed 0·40 or less to the overall amplitude of these cycles. Despite the decreased intensity of the cough portion, amplitudes of those cycles containing coughs averaged 1·25 times greater than that of adjacent cycles at 48 h and 1·68 times their height at 72 h.

Lethal exposure to $ZnSO_4$: Bluegill ventilation and cough frequencies increased steadily after the start of exposure to 10 mg $ZnSO_4$ litre^{-1} until, between 8 and 24 h,

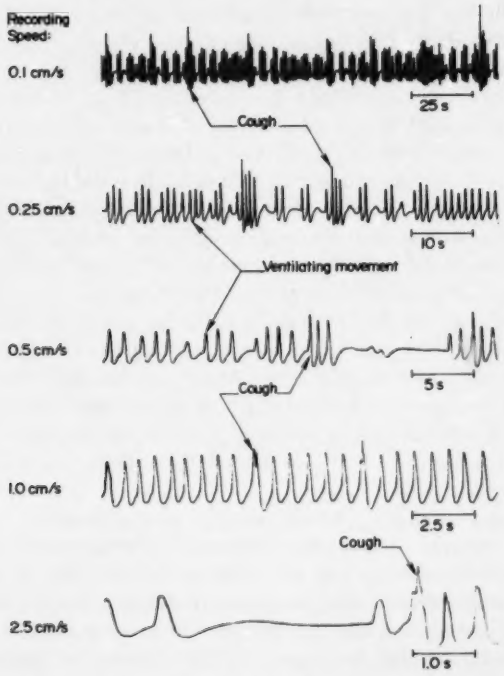

Fig. 4. Respiratory pattern of a bluegill at 72-h exposure to 3·0 mg $ZnSO_4$ litre^{-1} recorded at different paper speeds.

they peaked at 76 and 17·6 min^{-1}, respectively (Table 1). At some point in the exposure each fish would begin to ventilate continuously, and, when this occurred, the respiratory movements were hardly distinguishable from one another if strip-chart recorder paper speed was set at 0·1 cm s^{-1} (Fig. 5). Separating the breathing signals from each other by operating the recorder at faster paper speeds showed that the respiratory cycles had become smoother and more uniform and that the respiratory pattern was regularly punctuated with coughs. These conditions prevailed until just before death. At this time the respiratory signals became erratic, coughs were more difficult to detect even when present, and it appeared that ventilation and cough frequencies began to decline, although counts were not made for these.

Apneic periods disappeared from three of the fish by 4 h exposure and from all of the fish between 4 and 8 h exposure, but had not increased or decreased substantially before doing so. At the end of the 4-h exposure, however, the mode for

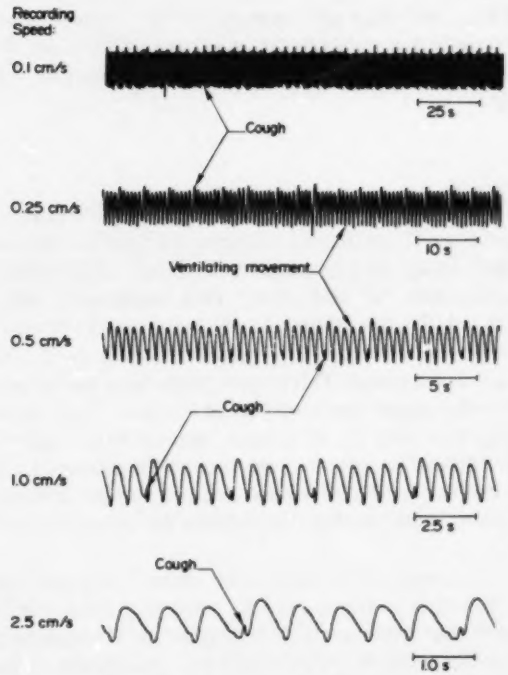

Fig. 5. Respiratory pattern of a bluegill at 24-h exposure to 10 mg $ZnSO_4$ litre^{-1} recorded at different paper speeds. Signal polarity reversed throughout.

the number of ventilating cycles in each group succeeding an apneic period increased to four. Coughs were not found in any group that had less than three cycles and, of 129 groups of ventilating cycles examined from six fish, 86% contained coughs.

As was true for fish exposed to sublethal $ZnSO_4$, respiratory movements occurred more quickly in fish exposed to lethal zinc than in acclimated fish (Table 2). The cough occupied from about one-seventh to one-sixth (0·14–0·17) of the time involved in an entire ventilatory cycle. During the 48-h exposure cough amplitudes decreased dramatically and the means calculated for the different recording periods fluctuated between 0·29 and 0·43 of the total change in potential. Also decreasing was the relative amplitude of these cycles; at 8-h exposure cough-containing cycles closely approximated neighbouring ventilatory cycles and averaged only 1·02 times greater in amplitude. Despite the decreasing amplitude of the cough portion within ventilating cycles, the overall depth of ventilation increased substantially during

exposure to $ZnSO_4$. Although not measured, this change was evidenced by the necessity to attenuate the respiratory signal periodically so that the tracings remained within a 1- to 4-cm swing of the recording pens for maximum readability of the recordings.

Recording difficulties

In these and past tests recognition of coughing manoeuvres on strip-chart recordings ranged from the extremely simple to the next-to-impossible. Generally, coughs were most easily distinguished from other ventilatory movements on recordings of acclimated fish and of fish that displayed a definite respiratory response to a toxicant. Besides enhanced amplitude, coughs in acclimated fish were often followed by two or more fairly rapid ventilating cycles that occasionally occurred as quickly as the cough. This pattern imparted a banded appearance to the records when recorder paper speed was set at $0{\cdot}1\ cm\ s^{-1}$ and consequently made cough counts very easy (Fig. 2). In severely stressed fish coughs were still easily discerned on the basis of amplitude, even though amplitude of cough-containing cycles declined relative to adjacent cycles. Because amplitude was consistent from cycle to cycle in hyperventilating fish, the slightest deviation from normal amplitude was easily seen.

Most difficult for cough recognition were those polygraph tracings recorded during the first part of exposure (especially sublethal), from fish facing imminent death, and from fish that predominantly displayed type II coughing manoeuvres. Of the several factors responsible for this difficulty, locomotor activity and loss of signal were most important. Often the calm, well-acclimated fish would become hyperactive just after the start of exposure, and the tracing of respiratory movements would become almost obliterated because of the detection of rapid and randomly fluctuating biopotentials during turning around manoeuvres, pectoral fin activity, and body undulations. Such recordings were scanned for only the large coughing movements as betrayed by greatly increased amplitude. However, a quick tail flick or violent head shake would at times produce a signal that looked much like a large amplitude cough, and the segregation of these counterfeit coughs from actual coughs was difficult. Fortunately, periods of hyperactivity usually became more sporadic as exposure continued and the record became more manageable.

Loss of signal amplitude plagued several test runs, although it was generally not a problem when monitoring fish in control water. After the start of exposure, however, reduction in signal amplitude frequently occurred, and, in some cases, the signal would disappear altogether. With $ZnSO_4$ this problem was usually short-lived, lasting only a few hours, after which signal amplitude would regain or surpass that of control levels. One of the known causes, the fish changing its position in relation to the electrodes (Heath, 1972), was ruled out as a possible cause of the difficulty because the longitudinally designed chambers maintained the fish in a position

almost always directly between the electrodes, and these were not further apart than 1·5 times the length of the fish for maximum detection of the signal. Other possibilities included an upset of ionic equilibrium at the electrode–water interface due to the addition of metallic salts ($ZnSO_4$ in this case), alteration of the signal-propagating features of the test water because of altered conductivity, or a reduction in signal output from the fish as it first sensed the toxicant. The exact cause was not determined, and attempts at increasing signal amplification often resulted in the addition of unacceptable noise that tended to produce erratic tracings and further obscure respiratory movements. Provided the signal did not disappear altogether, large coughing movements could still be discerned.

Despite these problems certain remedial steps improved strip-chart recording quality and lessened interpretative difficulties. Paper speed was occasionally increased. Normally, recordings were made at 0·1 cm s^{-1}, a speed that was adequate for separating respiratory movements during normal ventilation as well as being a paper-conserving measure. In these tests, however, it was sometimes difficult to determine whether or not some of the movements were weaker (type II) coughs or merely somewhat more intense ventilating movements. In this event, 0·5, 1·0 and 2·5 cm s^{-1} were ideal paper speeds because ventilating movements and minor deviations within these movements were effectively separated. Also, respiratory events were more easily discerned on recordings in which the bioelectric potentials developed as previously described, i.e. in an upward, positive direction on the record. So long as the fish faced one electrode (presumably the active, or positive, electrode), biopotentials developed in this manner. Whenever the fish turned around in its chamber, however, the tracing became a mirror image of the described respiratory events. In addition, setting the preamplifier filtration at 30 Hz, or, occasionally, 10 Hz, removed any residual 60-cycle interference that remained after grounding drains, headboxes, waterbaths and the stainless steel wire shields for the lights. Consequently, coughing movements were not lost on blurred recordings. Coughing movements occurred as fast as 10–12 Hz, however, so 10 Hz was the lower limit for filtration.

DISCUSSION

The origin of fluctuating potentials detected with non-contact electrodes and associated with the respiratory movements of fish is not well understood. It is known that a potential difference develops between two electrodes submerged in water whenever the water is moved past one electrode more than the other. This occurs because water movement disturbs the electrochemical equilibrium of the electrode surfaces, and as long as one electrode is affected more than the other, it makes no difference whether the water movement is generated by stirring, moving the

electrode itself, or by a fish as it moves about or jets water from its opercula (Spoor *et al.*, 1971). Wave propagation to electrodes has been used to explain potentials recorded from gill-beating and tail movements in the aquatic salamander *Necturus maculosus* (Goodman & Weinberger, 1971) as well as the respiratory movements of fish (Pasztor & Kleerekoper, 1962; Kesler, 1972; Yakimovich & Bergman, 1977).

Another, more probable, explanation is that these potentials originate in the respiratory and other muscles of aquatic animals and are volume-conducted through the water to the recording electrodes (Camougis, 1970). There is some evidence to support this view. Polygraph recordings of heart and ventilation activity recorded by means of a unipolar electrode inserted into the ventral region of a fish show ventilating movements as smooth gradual changes in potential with the QRS complex of the electrocardiogram (ECG) superimposed on the tracing (Marvin & Heath, 1968; Cairns *et al.*, 1970). Except for the ECG component, these recordings look remarkably similar to the ventilating movements recorded here with non-contact electrodes and are suggestive of a summed volume-conducted potential in the body of the fish rather than the higher frequency, irregularly produced volleys of potential expected if the electrode were detecting action potentials of only the muscle fibres into which it was inserted. Because fish are thin-skinned and covered with low-resistance mucous, these potentials can be expected to propagate outwards into the conducting medium in which they live, and be sensed with remote receiving electrodes (Barham *et al.*, 1969). Predators with electric field sensing capabilities will often cue in on these bioelectric fields surrounding their prey (Kalmijn, 1979).

Remote electrodes can pick up human muscle action potentials as well. Dramatic recordings are made when a hand submerged between two electrodes is clenched and relaxed alternately as a fist (Camougis, 1970), and moving a finger back and forth produces a waveform not unlike that produced by the ventilatory movements of a fish (Heath, 1972). These movements displace little water so the recorded waveform is not the result of water movement past the electrodes. In fact, although stirring of the water with a paddle produces a varying potential between mesh electrodes, little effect is observed with singular wire electrodes, even when the stirring is violent or if water is spurted in a pulsing manner from a submerged tube directly at the electrodes (Heath, 1972).

It appears, then, that just as the electrocardiogram is the result of temporal and spatial summation of all the myocardial fibres (Geddes & Baker, 1968), the biphasic potentials recorded from a quietly resting fish represent a similar summation of the electrical activity occurring in all the head and branchial muscle fibres involved in ventilation. For this reason, and for lack of a term to describe the monitoring of this very specific type of activity, I proposed to call such recordings 'electrobranchiograms' (EBGs). If the procedures for recording and interpreting the EBG were then standardised, as has been done for the ECG, I believe many of the interpretative difficulties encountered while trying to make counts of coughing in fish would be eliminated. The designation of cough types described from EBGs recorded here

may provide a starting point for classifying components of the waveform, but guidelines are needed to ensure that, whenever and wherever recorded, the EBG is an accurate and faithful reproduction of the waveform produced by the breathing and coughing movements of fish.

Previously, fish coughs have been classified according to the direction of water flow through the buccal and opercular cavities. Although gradations existed, coughs occurred in two primary patterns: forward and backward. In forward coughing the net expiratory flow of water is out of the fish's mouth, in contrast to backward coughing in which most of the water leaves via the opercular openings (Hughes & Adeney, 1977). Hughes (1975) further subdivided backward coughing into two categories, weak and strong.

A weak cough consisted of a greater than normal expansion and contraction of the buccal and opercular cavities, particularly the buccal. This action produced a higher than normal positive pressure in the buccal cavity and resulted in a forceful movement of water across the gills in the normal direction. A strong cough occurred whenever the pressure gradient between the buccal cavity developed a high, uncharacteristic negative pressure relative to the opercular cavity, causing water flow to be reversed across the gills. The net flow might still exit via the operculum, however, dependent on the position of the lower jaw, which determined whether the mouth was opened or closed during the reversal phase. The reversal phase was quickly followed by a prolonged, high positive pressure in the buccal cavity, after which normal pressure cycles were resumed. The entire cough occupied one-quarter to one-half of a normal ventilatory cycle and, in a strong cough, produced a two- to five-fold increase in differential pressure across the gills during the reversal phase.

The sunfish used in this study displayed three basic cough patterns, or variations thereof, and no attempt was made to categorise these as either forward or backward coughs. To do so would require extensive and simultaneous monitoring of pressure changes in the buccal and opercular cavities, jaw and opercular movements, and electromyographic (EMG) activities within the main respiratory muscles. Nevertheless, similarities were noted between bluegill coughs recorded on EBGs and those classified according to EMG and manometric studies of trout. Type I coughs corresponded closely to forward, which are high intensity coughs, or strong backward coughs, i.e. large fluctuations in biopotential as determined from the EBG compared with large fluctuations in buccal pressure and EMG activity (Hughes & Adeney, 1977). Type II coughs, on the other hand, were more similar to the weaker backward coughing movements wherein Hughes (1975) detected only an extra burst of activity on the EMGs of the adductor mandibulate and its synchronous muscles. No obvious counterpart was seen for Type III coughs, but when observed visually they looked like weak backward coughs.

The different breathing and coughing patterns observed in bluegills exposed to $ZnSO_4$ may have as many toxicological implications as do increased breathing and coughing frequencies. Whereas eupnea, continuous and rhythmic breathing, is the

normal condition in man, the norm for bluegills is a form of respiratory arrhythmia similar to that termed Biot's breathing. Seen also in well-aerated Port Jackson sharks *Heterodontus portusjacksoni* (Capra, 1975), Biot's breathing is an abnormal condition in man wherein apneic periods alternate with groups of breathing cycles that diminish in amplitude from first to last. In well-acclimated bluegills the first ventilating cycle after apnea was not always the largest, but the overall appearance of the EBG was still remarkably similar.

During toxicant challenge, however, the bluegill respiratory pattern underwent several changes, some of which were only transitory and seemingly dependent on the extent and length of exposure. At sublethal concentrations coughing and breathing frequencies commonly increased, peaked, and began to decline until the established base rate was, or nearly was, attained in a relatively short time (24–96 h). Variable between fish in this study, the respiratory pattern reflected these changes in one of three ways. Sometimes the number of ventilating cycles increased in each group as ventilation rate increased after the start of exposure, and, as cough rate

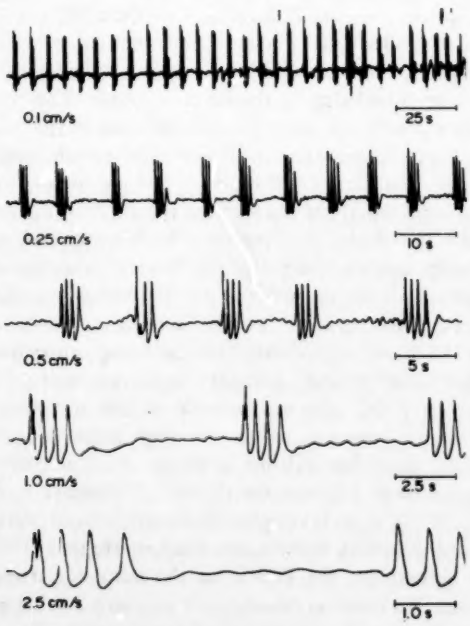

Fig. 6. The regular, blocked appearance of the respiratory pattern often seen on bio-potential recordings of the bluegill during sublethal exposure and the beginning stages of lethal exposure to $ZnSO_4$. This fish was recorded at 4-h exposure to 10 mg $ZnSO_4$ litre^{-1}.

went up, coughs appeared in more and more of the ventilating groups until they occurred in every group at the peak of response. Because ventilating cycle duration decreased, more cycles were present per minute without a substantial, concomitant decrease in duration of the apneic periods. In addition, cycle amplitude became more consistent within a group, and all these changes imparted a very regular and blocked appearance to the EBG recording (Fig. 6). Duration of the apneic periods decreased in other fish, as ventilation and cough frequencies rose until the fish began hyperventilating at the peak of response, but not so rapidly that type III coughs were predominant. This produced a uniform breathing pattern regularly interspersed with coughs, usually of type I or II (Fig. 7). Lastly, sometimes cough rate increased without a correspondingly large change in ventilation frequency. Apnea remained about the same and the resultant EBG had a very irregular appearance (Fig. 8). As the decline in response occurred, these patterns gradually reversed and resumed their former appearance typical of acclimated fish.

In fish exposed to lethal toxicant levels cough and ventilation frequencies

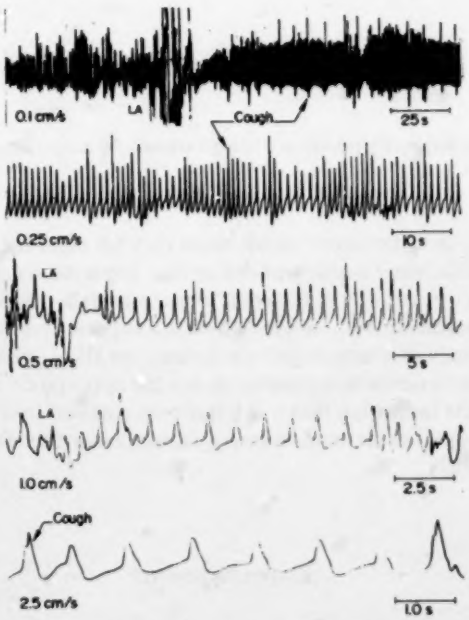

Fig. 7. Pattern of hyperventilation of a bluegill exposed for 1·5 h to 3·0 mg $ZnSO_4$ $litre^{-1}$. LA is locomotor activity.

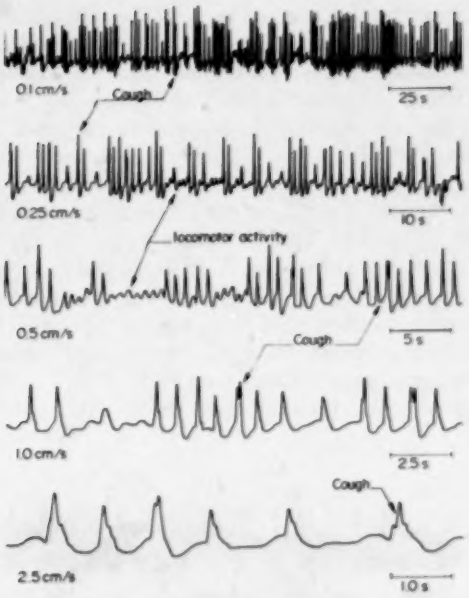

Fig. 8. Irregular respiratory pattern of a bluegill exposed for 3 h to 10 mg $ZnSO_4$ $litre^{-1}$.

increased steadily until just before death, when they fell off as the fish was expiring. Bluegills exposed to lethal concentrations of zinc displayed transitory respiratory patterns similar to those seen at sublethal levels (especially the blocked pattern of Fig. 6), but these terminated without exception in a eupneic-type ventilation (Fig. 5). During these periods of intense hyperventilation type III coughing predominated, and it leaves open to conjecture whether or not the appearance of this respiratory pattern is a definite indication that a fish has been exposed to a potentially lethal concentration of a particular toxicant even if exposure was discontinued and the fish recovered completely.

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